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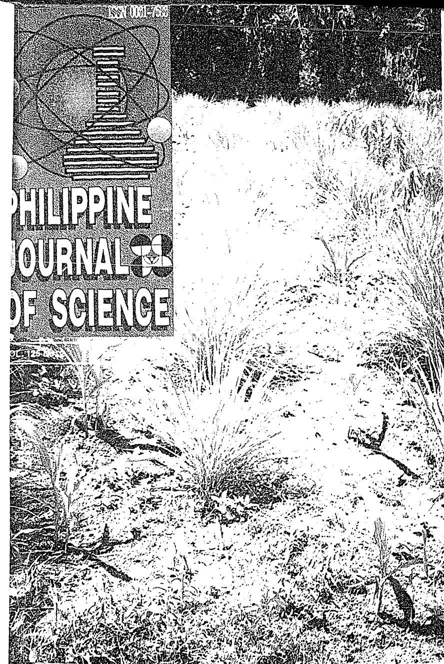

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OUR COVER. The cover photo, taken from the fifth manuscript published in this issue, is a picture of lemongrass plantation in Nagcarlan, Laguna intercropped with corn and papaya.

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PREPARATION OF SLOW RELEASE MICROCAPSULES CONTAINING NUTRIENT BY FLUIDIZED BED COATING METHOD

YUJI YOKOTA[1], JOSIE L. PONDEVIDA[2] and CECILE F. ALFONSO[2]

ABSTRACT

Preparation of nutrient containing microcapsules by fluidized bed coating method was studied. The process involves granulation of the raw materials and the spray coating by Wurster process.

Granules were prepared from powdered rice husk, ammonium sulfate as nutrient and soluble starch as binder. Two types of coating process, the Top and Bottom Spray coating, was studied. The Bottom spray method exhibits higher coating efficiency than the Top Spray method. Using the Bottom Spray method, effect of coating material and the amount of coating on the NH_4^+ release of coated granules was determined. Dissolution test of the microcapsules for 60 days period was conducted. Granules coated with Ethylcellulose exhibits slower dissolution rate compared with the granules coated with polystyrene.

INTRODUCTION

Encapsulation is the unique packaging process which is arousing broad interest in many fields. Both liquids and solids may be encapsulated in sizes ranging from a few microns to several thousand microns in diameter and in wide variety of shell materials. The capsules may be of use for a number of reasons. These include: (1) protection of reactive materials from their environments prior to use, (2) safe and convenient handling of materials which are otherwise toxic or noxious, (3) means of providing controlled, sustained release of materials following application, and (4) means of handling liquids as solids.

Characteristic common to practically all capsules is that the capsule contents are ultimately released. Release mechanisms which may be built into the capsule include thermal release, mechanical rupture, dissolution of the shell, biodegradation of the

[1]Hokkaido National Research Institute
2-17-2 Tsukisamu-Higashi, Toyohira-Ku, Sapporo, 062 Japan
[2]Industrial Technology Devt. Institute
Bicutan, Tagig, M.Manila, Philippines

shell, and controlled permeation or diffusion. Frequently, combinations of these release mechanisms are desirable.

During recent years, encapsulation technology has made great advances with a corresponding increase in interest in capsule utilization. The cost of encapsulation, although not prohibitive in many cases, is a factor in determining feasibility of using this form of packaging. In general, encapsulation is practical in cases where it will do a job which cannot otherwise be done or do the job better. Where safety and/or convenience justifies its added cost, the cost of encapsulation is negligible compared to the value of the encapsulated product or where encapsulaten enhances the value of the product.

Microencapsulation process is effective in afforestation technology for soil improvement and fertilization. The controlled release of nitrogen and other nutrients in fertilizer is an important aspect in the field of agriculture.

In the previous paper (Yokota et al. 1994), preparation of slow release microcapsule using ammonium sulfate (AS) or activated carbon with ammonium sulfate (ACAS) as core material investigated. Ethylcellulose was used as coating material in the complex emulsion method. In this method, the nutrient contained in the microcapsules are limited. Therefore, studies were undertaken to increase the nutrient content of the microcapsules and improve its performance.

In this paper, microcapsules were prepared by the top and bottom spray coating method in a fluidized bed apparatus. The effect of different coating materials and the amount of coating on the NH_4^+ release were discussed from the view point of determining the release pattern of the microcapsules.

MATERIALS AND METHODS

Microencapsulation process involves granulation of raw materials and encapsulation by spray coating method. Granulation process involves selection of suitable concentration of the binder. For the encapsulation process, the conditions studied were spray coating method, type of coating material and amount of coating. Figure 1 shows the flow diagram of microencapsulation process by fluidized bed coating method.

Preparation of Granules

Granulation experiments was conducted for the preparation of core materials for coating process.

Powdered rice husk and ammonium sulfate were weighed with a ratio of 9:1. Five hundred (500) grams of the raw materials were mixed thoroughly in the kneader (Fuji Paudal Co. Ltd.). About 320 ml of 10% (w/v) soluble starch solution was sprayed uniformly to the mixture in the kneader until the desired consistency of the mixture was attained. The mixture was then passed through the extruder (Fuji Paudal Co. Ltd.) and finally granulated in the marumerizer (Fuji Paudal Co. Ltd.) for 20-30 seconds. The granules produced were allowed to dry at room temperature for five (5) hours and drying was completed in an oven at 65°C for 24 hours. Dried granules were then sieved

and the granules which size ranging from 0.5 to 2.0 mm were collected and subjected for the coating process.

Preparation of Coating material

The measured amount of the coating material i.e. ethylcellulose or polystyrene was dissolved in dichloroethane producing a concentration of 10% w/v.

Spray Coating Method by Fluidized Bed Apparatus

The physical coating method of microencapsulation was carried out using top and bottom spray in the fluidized bed as described below:

Top Spray Method

Figure 2 shows the Top Spray coating apparatus. The reactor is made of stainless steel and 18.5 cm I.D. and 50 cm in height. The lower portion of the reactor (10 cm I.D.) is tapered and the bottom is equipped with a gas distributor. An adjustable spraying nozzle is located at the middle portion of the reactor.

One hundred (10) grams of the uncoated granules were fed into the vessel. Air is blown into the reactor at a controlled velocity (1.22 - 5.00 m /min) to fluidize the sample. Coating solution was applied into the fluidizing granules by a microtube pump through the spraying nozzle with spraying pressure of 1-2 kg/cm at feed rate of 220 ml/hr. Coating process was carried out during the circulation of the granules around the spraying nozzle in the fluidized bed at the temperature of 35 - 40°C. The solvent of the coating solution eventually vaporized and film was formed on the surface of the granules. As the coating process was completed, the remaining solvent in the coated granules was evaporated by continuous introduction of air to the vessel for about 30 minutes.

Bottom Spray Coating

Figure 3 shows the bottom spray coating apparatus. The Bottom Spray method was carried out in the same equipment but the reactor is made of high grade acrylic resin cylindrical tube. The spraying nozzle is attached at the bottom portion of the reactor which is provided with a partition. The gas distributor is designed in such a way that the size of the perforation is bigger at the inner portion than the outer portion of the plate.

One hundred (100) grams of uncoated granules were fed into the reactor and air was injected at a controlled velocity to fluidize the sample. Measured amount of coating solution was pumped into the nozzle by a microtube pump and sprayed into the fluidizing granules.

Dissolution Test for NH_4^+ Release

One half (0.5) gram of microcapsule was added to one (1) liter of distilled water, with the pH initially adjusted to 7.0. The sample was placed in an incubator maintained

at 30°C. Five (5) ml aliquot taken at regular time interval was filtered and the NH_4^+ concentration was determined by Automated Phenate Method using the Technicon Auto Analyzer II.

RESULTS AND DISCUSSIONS

Microencapsulation Process

Fluidized bed apparatus has two types of spray coating process, one is the Top Spray and the other one is the Bottom Spray. Both methods were studied to determine the most suitable coating process in the production of microcapsules with respect to NH_4^+ release. The same operating conditions such as spraying velocity and pressure, air velocity and bed temperature were used in both methods.

For the Top Spray method, the coating solution was sprayed from the top of the fluidizing granules. Rapid evaporation of the solvent in the coating solution was observed before it reaches the lower portion of the vessel. The solvent loss resulted to the increase of concentration and sudden drying of the coating solution. As the coating solution dries, film-like particles developed on the walls of the vessel instead of coating the fluidizing granules in the vessel. Therefore, the amount of coating solution consumed does not completely coat the granules but carried out by the exhaust gasses.

On the other hand, for the bottom spray coating method, the coating solution was sprayed from the bottom of the vessel. The opening of the gas distributor has variable sizes to enhance the circulation of the granules. During the coating process, there is instantaneous contact of the coating solution and granules. It was found that the granules was immediately coated and there was a continuous circulation of the particles around the spraying nozzle. The granules were coated repeatedly during the process and uniform build-up of coating in the granules was attained at the run progressed. Besides, it was observed that simultaneous drying of the coating solution in the granules occurred during the process resulting to less agglomeration of the particles.

Experiments were conducted using the Top and Bottom Spray Coating Methods with 10% ethylcellulose solution as coating material. The percentage increase on the weight of the granules from the Top Spray Coating Method is 10% while in the Bottom Spray Coating Method is 75%. This indicated that the amount of coating obtained from the Bottom Spray Method is relatively high compared to the Top Spray Method. Photo 4b and 5b show the difference of coating of the microcapsules produced by Top and Bottom Spray method.

Analysis

Scanning Electron Microscopy (SEM)

Granulated sample and microcapsules coated with 10% Etcell solution produced from Top and Bottom Spray methods were examined with Scanning Electron Micros-

copy. Photo 1 shows the SEM photograph of uncoated granules appears to be rough and in Photo 1b, tiny spaces between the particles are visible.

Photo 2 shows the SEM photograph at 35X magnification of the microcapsules produced. Photo 2a & 2b show that the microcapsules produced from Bottom Spray method has smoother surface than the microcapsules produced from Top Spray method. The appearance of the surface with respect to the amount of coating dependent upon the system of coating used.

Photo 3 shows the SEM photograph of microcapsules taken at 1000X magnification. The photographs describe the pore structures of the coated granules. The pore structure of the coated granules from Top Spray method varies from that of the coated granules by Bottom Spray Method. The pores in Photo 3b is very much larger than in Photo 3a. This indicates that the granule from Top Spray has less coating compared with the granule from Bottom Spray.

Dissolution Test

Microcapsules containing NH_4^+ prepared from the Top and Bottom spray methods are evaluated. Dissolution test was conducted to determine the rate of NH_4^+ release.

Effect of Coating on the NH_4^+ Release of the Product

The amount of NH_4^+ release for a certain period was measured to determine the dissolution characteristics uncoated granules and microcapsules coated with ethylcellulose and polystyrene using the Top Spray Method.

Figure 4 describes the effect of coating material on the NH_4^+ release of the microcapsules. It is shown that the uncoated granules exhibit faster dissolution rate compared to the coated granules. However, 80% of the NH_4^+ contained in the granules coated by both ethylcellulose and polystyrene were released within two (2) days. The results showed that the coating of the granules produced from the Top Spray method is still insufficient.

Effect of Coating Method on the NH_4^+ of the Product

The release pattern shown in Figure 5, describes the dissolution of NH_4^+ from the microcapsules coated with ethylcellose by top and bottom spray coating methods. Results indicate that 80% of the NH_4^+ contained in the coated granules from Top Spray was released within two (2) days while the granules from Bottom Spray, released 70% of the NH_4^+ content within 15 days. This indicated that the method of coating has significant effect in the NH_4^+ release of the microcapsules. Likewise, it shows that the Bottom Spray method is more efficient and suitable for producing slow release microcapsules compared with the Top Spray Method.

Effect of the Amount of Coating on the Dissolution of the Product

Using the bottom spray coating, a study on the degree of coating of the granules was undertaken. Microcapsules produced has 7% and 20% coating (weight increase of

uncoated granules) of ethylcellulose. Figure 6 shows the effect of the amount of coating of the granules on the NH_4^+ release within 60 days period of dissolution test. It can be seen that the microcapsules having 7% coating of ethyl cellulose rapidly release 60% of NH_4^+ content within 5 days and the remaining NH_4^+ was gradually released within 60 days. On the other hand, for microcapsules with 20% coating of ethylcellulose, released 75% of the NH_4^+ content gradually for 60 days. Results of the analysis explains that the dissolution rate is greatly influenced by the amount of coating materials in the microcapsules.

Effect of Coating Material on the Dissolution of the Product

Another experiment on the Bottom Spray Method using polystyrene was conducted. Microcapsules produced has 20% coating of polystyrene. Figure 7 illustrates the release pattern of the microcapsules with 20% coating of ethylcellulose and polystyrene for a period of 60 days. It was observed that within 10 days, ethylcellulose coated granules had released 50% of its NH_4^+ content while the polystyrene coated granules released 60%. Results indicated that using ethylcellulose coating is more effective compared with polystyrene because microcapsules coated with ethylcellulose exhibits slower dissolutions rate than the one coated with polystyrene.

CONCLUSION

The objective of this study was to prepare microcapsules by Fluidized Bed method and determine the NH_4^+ release pattern of the microcapsules with different coating material.

The application of the bottom spray coating process in fluidized bed was found to be more efficient and suitable for production of slow release microcapsule. Bottom Spray method was found to be more economical than the Top spray method because of the following reasons:

1. the 80-90% of the coating solution consumed was coated to the granules.

2. simultaneous coating and drying occurs during the process.

3. less chances of agglomeration of the particles occurs.

The type and the amount of coating material in the microcapsules affects the dissolution rate of the microcapsules. Ethylcellulose coating material shows better performance than polystyrene; slower NH_4^+ released to water of ethylcellulose coated granules was observed. Increasing amount of coating resulted to slower dissolution rate of microcapsules.

Further studies on the other types of coating material, increased amount of coating and the optimum processing conditions will improve the quality of the microcapsules and increase possibilities for release retardation will be obtained.

ACKNOWLEDGMENT

This study was made possible thru the Technical Cooperation Program of the Institute for Transfer of Industrial Technology (ITIT), with the Hokkaido National Industrial Research Institute (HNIRI) Japan and the Department of Science and Technology (DOST), Philippines.

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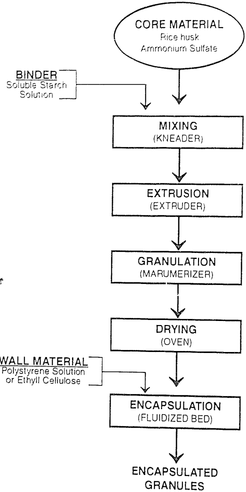

Figure 1. Flow Diagram of Microencapsulation by Fluidized Bed Method.

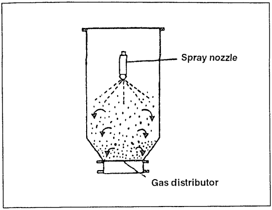

Figure 2. Top Spray Coating Apparatus.

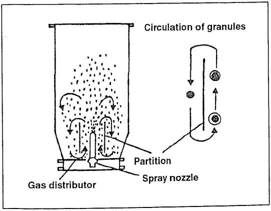

Figure 3. Bottom Spray Coating Apparatus.

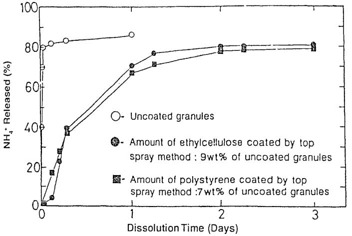

Figure 4. The percentage of NH_4^+ released from microcapsules.

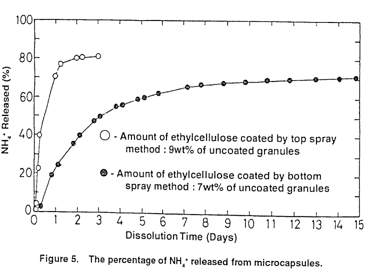

Figure 5. The percentage of NH_4^+ released from microcapsules.

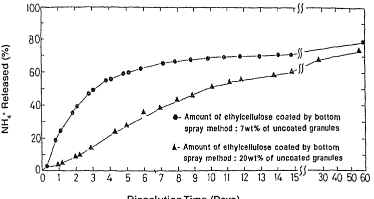

Figure 6. The percentage of NH_4^+ released from microcapsules.

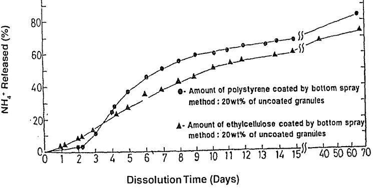

Figure 7. The percentage of NH_4^+ released from microcapsules.

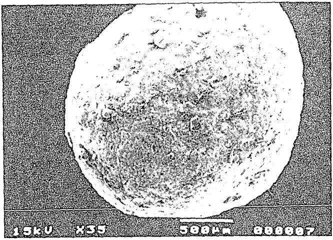

Photo 1a. Uncoated Granule at 35X Magnification.

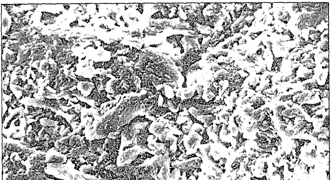

Photo 1b. Uncoated Granule at 1000X Magnification

Photo 1. SEM Photographs of Uncoated Granule.

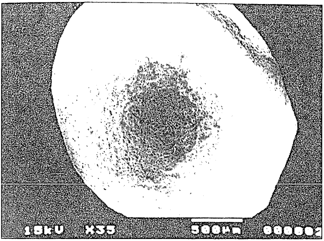

Photo 2a. Coated Granule from Top Spray Method at 35X Magnification.

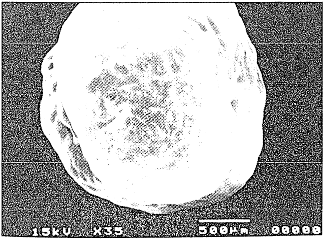

Photo 2b. Coated Granule from Bottom Spray Method at 35X Magnification.

Photo 2. SEM Photographs of Granules Coated with Etcell Solution.

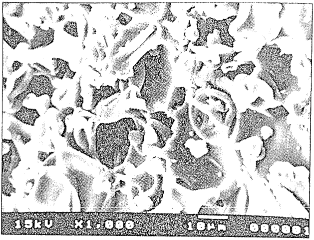

Photo 3a. Coated Granule from Top Spray Method
at 1000X Magnification.

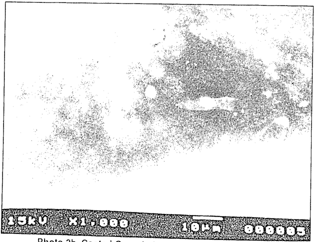

Photo 3b. Coated Granule from Bottom Spray Method
at 1000X Magnification.

Photo 3. SEM Photographs of Granulese Coated with Etcell Solution.

RIBOFLAVIN REQUIREMENTS OF SOME FILIPINO LACTATING WOMEN

JUANITA R. MADRIAGA, JUANITA M. MARCOS, and LEAH A. PERLAS
Nutritional Biochemistry Division
Food and Nutrition Research Institute
DOST Complex, Gen. Santos Avenue, Bicutan, Taguig, M.M.

ABSTRACT

The study was conducted on 11 lactating women to validate the riboflavin recommended allowance of 0.6 mg/1000 kcal. The women, who were riboflavin deficient (EGR-AC $\geq$ 1.3) at the start of the study were fed 4 levels of riboflavin diet at 8-days interval for 32 days while living in their own homes. Riboflavin intakes were increased to 0.5, 0.6 and 0.7 mg/1000 kcal using riboflavin supplements given in gelatin capsules during the 2nd, 3rd and 4th levels. Improvement in their blood riboflavin levels (EGR-AC) was measured at the end of each dietary level. Based on the regression of EGR-AC on the riboflavin intake, the estimate of minimum requirement ranged from 0.47 to 0.69 mg/1000 kcal with a mean of 0.60 $\pm$ 0.07 mg/1000 kcal. The RDA for the lactating mothers was calculated by adding 2 standard deviations to the mean to cover the 97.5% of the population. This resulted to a value of 0.74 mg/1000 kcal or 1.6 mg/d, slightly higher than the 1989 recommendation of 1.4 mg/d but is deemed sufficient to achieve a normal riboflavin status as indicated by EGR-AC values of 1.29 and below for free-living lactating women.

INTRODUCTION

Vitamin supplementation for pregnant and lactating women is a precautionary measure not only to ensure an adequate intake of vitamins by the infants but also for the maintenance of the mother's own nutritional status. The concentration of riboflavin in human milk samples was shown to be dependent on the amount of riboflavin supplementation of the mothers during the period from two to twelve weeks after delivery.

The US Food and Nutrition Board (1989) recommends an additional daily intake of 0.5 mg/d for the first 6 mo of lactation and 0.4 mg thereafter. These recommendations were based on an assumed mean milk riboflavin content of 35 ug/dL, average milk production of 750 and 600 mL/d during the first and second 6 mo of lactation, respectively; 70% utilization of riboflavin for milk production and coefficient of variation of milk production of 12.5%. In Australia (1987), the additional amount of 0.8 mg/d during lactation allows for the additional energy requirement and for that secreted in milk. In Canada (1990), the recommended increment for lactation is 0.4 mg/d above that for non-lactating women. These recommendations are higher than that provided by the increase in energy requirement proposed by FAO/WHO (1967). The Philippine RDA during lactation is 0.4 mg/d above that for non-lactating women. This was based on an allowance of 0.6 mg/1000 kcal in the absence of local studies on riboflavin requirement of lactating women (RDA, 1989).

This study aims to validate the above cited RDA by determining the riboflavin intake required to achieve an erythrocyte glutathione reductase activity coefficient (EGR-AC) of <1.3.

MATERIALS AND METHODS

Subjects

Eleven lactating mothers from a community in Barangay 765, San Andres, Manila participated in the study. These same women served as subjects in the protein requirement study following the multilevel N balance assay. All subjects belonged to the low socio-economic group. They lived in their homes and continued with their usual household chores while the study was being conducted for a period of 32 days. However, they were requested to eat their meals (breakfast, lunch, dinner) including morning and afternoon snacks at a designated house in the community where all the foods were individually weighed and cooked. Feeding was supervised by a dietitian to ensure that all the foods were consumed.

Diet

Isocaloric diets (2470 kcal) providing four levels of protein and riboflavin given at intervals of eight days were prepared from food items usually consumed (Villanueva et al., 1990). The riboflavin content was raised to 0.5, 0.6 and 0.7 mg/1000 kcal using synthetic riboflavin (USP) on the 2nd, 3rd and 4th levels, respectively. The synthetic riboflavin were given in gelatin capsules and taken everyday before breakfast.

Chemical Analysis

A composite sample for each subject was prepared for each level. The protein and energy contents of the diet were determined by the micro *kjeldahl* method and adiabatic bomb calorimetry, respectively. Riboflavin and thiamin contents of the diet were analyzed using the AOAC method for vitamins (AOAC,1965). Other nutrients such as iron and ascorbic acid were also analyzed.

Biochemical Analysis

Venous blood samples were drawn initially for baseline data and at the end of each 8-day period for the determination of hemoglobin (ICSH,1978), hematocrit by micro capillary method, total serum protein, serum albumin (Debro et al.,1951) and EGR-AC (Sauberlich et al.,1972).

Statistical Analysis

Regression analysis of EGR-AC on riboflavin intake (mg/1000 kcal) was done for each subject. The riboflavin intake needed to attain an EGR-AC of < 1.3 was taken as the minimum requirement.

RESULTS AND DISCUSSION

The pre-study characteristics of the lactating mothers are shown in Table 1. Their ages ranged from 22 to 37 years with a mean ± standard deviation of 28.8 ± 5 y. The mothers were nursing their infants between 4 to 10 weeks with a mean of 7 ± 1 week. Body weight for height of the mothers ranged from 87.1 to 113.2 % of standard (FNRI,1975) with a mean of 99.4 ± 9.4%. Hemoglobin (13.1 ± 1.2 g/dL) and hematocrit (37.2 ± 2.9 %) were normal while serum albumin was deficient in two subjects (Tess and Rea). EGR-AC ranged from 1.35 to 1.66 which indicated that all the mothers were riboflavin deficient on admission. Higher EGR-AC values reflect greater riboflavin depletion (Campbell et al., 1990).

The nutrient composition and adequacy of the basal diet are shown in Table 2. As designed, energy intake of the basal meals met the recommended dietary allowance while protein intake was only 65.6 % of the RDA. All the other nutrients were below RDA levels. Based on the third national nutrition survey in 1987, lactating mothers had a very low riboflavin intake of 0.49 mg/d equivalent only to 36.8 % of the RDA. Likewise, the energy, protein, iron, thiamin and ascorbic acid intakes of these lactating mothers were all inadequate (Villavieja et al.,1989). It has also been observed that in communities where little meat or dairy products are consumed, riboflavin intakes are extremely low and are likely to be grossly inadequate specially when coupled with stress due to pregnancy and lactation (Bates et al.,1981).

Table 3 shows the relation between mean riboflavin intake and mean EGR-AC values for the lactating mothers at different periods of the study. As analyzed, the mean riboflavin intake during the first 8-days was 0.30 ± 0.05 mg/d, coming solely from the food consumed. The intake represented only about 21.0% of the RDA. The mean riboflavin intake was increased to 1.10 ± 0.10 mg/d with the introduction of a 0.5 mg/1000 kcal riboflavin supplement, but the EGR-AC remained deficient. Only two subjects (Mercy and Baby) attained normal EGR-AC levels (< 1.3) when the mean riboflavin intake was raised to 1.35 ± 0.08 mg/d at the 3rd level. All the other subjects attained normal EGR-AC at the 4th level with an average riboflavin intake of 1.61 ± 0.10 mg/d. Other investigators (Bates et al., 1981) who employed longitudinal supplementation trials in villages reported higher EGR-AC values ranging from 1.3 to 1.5 even when supplementation increased the intake to 1.5 mg/d in Gambian women. Bates et al. (1982) reported that a daily intake of about 2.5 mg of riboflavin is sufficient to achieve biochemical normality in most lactating Gambian mothers.

The relationship of protein intake to EGR-AC is shown in Table 4. As protein intake increased, EGR-AC decreased tending to normalize. Rutishauser (1982) reported that riboflavin and protein appear to be mutually limiting factors so that if protein intake is inadequate, riboflavin is not utilized or vice versa. When the nitrogen balance is positive more riboflavin is retained. In this study, the EGR-AC did not normalize even during the third period when the protein intake was positive (Villanueva et al., 1990) and riboflavin intake was 1.35 ± 0.08 mg/d. In a study done in India, it was observed that a daily riboflavin supplement of 4 mg given for one month failed to normalize the EGR-AC in half of the subjects. This finding was attributed to inadequate protein and energy intakes which impaired vitamin supplement utilization (Bamji et al., 1979). The lactating women in this study were not confined in the metabolic ward but stayed in their homes in the community and were engaged in their

usual activities and household chores. These activities could have served as stressor factors which increased their nutrient requirements. A study on Gambian lactating women (Bates et al., 1981) showed increased energy expenditures with increased riboflavin requirements. It was possible that they needed more riboflavin to achieve normal levels.

The regression of EGR-AC on the riboflavin intake (Figure 1) and the estimate of riboflavin requirement on the 11 lactating mothers are shown in Table 5. The estimates of minimum requirement ranged from 0.47 to 0.69 mg/1000 kcal with a mean of 0.60 ± 0.07 mg/1000 kcal. A coefficient of variation (CV) of 12% was obtained. This value is much lower than those reported by Olson (1987) and Olson and Hodges (1987). It is also lower than the CV obtained in requirement studies on Filipinos (Madriaga et al., 1994; Perlas et al., in press; Kuizon et al.,1992). The RDA for Filipino lactating women was estimated to be 0.74 mg/1000 kcal or 1.6 mg/d after adding two (2) standard deviations of 0.60 ± 0.07 mg/1000 Kcal to the mean riboflavin requirement obtained in this study to cover the 97.5% of the population. This is slightly higher than the 1989 recommendation of 1.4 mg/d for lactating women on the first six months of lactation.

During lactation, the requirement is assumed to increase by an amount at least equal to that excreted in milk. At an average milk production of 750 mL/d for Filipino lactating women (Villanueva et al., 1990), about 0.26 mg riboflavin is secreted in milk and thus taken in by the infants. Unfortunately, there was no analysis done for breastmilk riboflavin in this study. The amount of 1.6 mg riboflavin per day for the lactating women took into consideration the added cost for lactation for the first six months. It may seem paradoxical that riboflavin status should improve spontaneously during lactation, despite the secretion of substantial amounts of riboflavin in human milk. From about the third month in lactation, EGR-AC was observed to decline approximately in parallel with the natural fall in milk output. At about this time the balance of supply and demand enables maternal body stores to begin to improve (Bates et al., 1981).

CONCLUSION AND RECOMMENDATION

The findings of this study indicate that an amount of 0.74 mg riboflavin per 1000 kcal or 1.60 mg/d for free-living lactating women was deemed sufficient to achieve a normal riboflavin status as indicated by EGR-AC values of 1.29 and below. In view of this finding, it is recommended that 0.74 mg/1000 kcal or 1.60 mg/d be adopted because this was experimentally determined from lactating women. Considering the vulnerability of this group of subjects (Kuizon et al.,1994) and the low dietary intakes of riboflavin, it is highly recommended that riboflavin supplementation be taken seriously. Foods rich in riboflavin such as milk and milk products, meat and meat organs, should be included regularly in their diets.

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Table 1. Characteristics of the lactating subjects on admission to the study.

Anthropometric and biochemical data	Subjects										
	ROSE	TESS	LOR	REA	LUCY	LUZ	TESS	FLOR	MERCY	BABY	ELI
Age (y)	26	27	26	28	23	31	35	35	22	37	27
Lactational age (wk)	7	4	7	6	7	4	8	10	8	8	9
Body weight (kg)	49.8	43.8	48.6	45.9	41.7	41.9	42.3	54.5	42.8	44.0	47.8
Height (cm)	145.5	139.5	144.5	152.5	146.5	149.5	153.0	157.0	150.0	145.5	154.5
Weight for Height (%)	113.2	109.5	112.2	94.5	93.4	89.9	87.1	106.3	91.8	100.0	95.8
Hemoglobin (g/dL)	14.5	11.7	13.8	14.5	13.4	11.2	13.6	13.6	12.4	11.7	14.2
Hematocrit (%)	41.2	34.5	38.8	41.0	34.2	33.8	37.3	38.0	35.2	34.5	40.7
Serum Albumin (g/dL)	3.98	2.04	2.92	2.21	2.95	3.78	3.58	3.58	3.46	3.69	4.50
EGR-AC	1.58	1.62	1.35	1.38	1.66	1.66	1.60	1.62	1.46	1.40	1.46

TABLE 2. Energy and nutrient composition and adequacy of basal diet for lactating women.

Diet Component	Philippine RDA	Level of component in diet	Adequacy (% RDA)
Energy, kcal	2202	2262	102.9
Protein, g	82	47.93	65.6
Riboflavin, mg	0.5/1000 kcal	0.823	72.8
Thiamin, mg	0.5/1000 kcal	0.689	62.6
Iron, mg	18	9.54	53.0
Ascorbic acid, mg	120	90.87	75.7

TABLE 3. Riboflavin intake (mg/d) and EGR-AC values for lactating women.

	Day on Study							
	Day 8		Day 16		Day 24		Day 32	
Subject	Ribo intake	EGR-AC	Ribo intake	EGR-AC	Ribo intake	EGR-AC	Ribo intake	EGR-AC
Rose	0.30	1.72	1.18	1.41	1.42	1.32	1.73	1.18
Tess	0.30	1.70	1.10	1.44	1.31	1.32	1.53	1.17
Lor	0.43	1.35	0.96	1.32	1.39	1.30	1.69	1.26
Rea	0.34	1.38	0.92	1.35	1.34	1.31	1.60	1.25
Lucy	0.29	1.53	1.11	1.36	1.31	1.31	1.53	1.22
Luz	0.29	1.55	1.11	1.37	1.31	1.31	1.53	1.26
Tess	0.29	1.45	1.11	1.38	1.31	1.30	1.53	1.20
Flor	0.22	1.73	1.29	1.40	1.55	1.33	1.81	1.17
Mercy	0.28	1.45	1.09	1.33	1.30	1.26	1.52	1.16
Baby	0.28	1.40	1.09	1.32	1.30	1.26	1.52	1.16
Bi	0.29	1.44	1.18	1.33	1.41	1.30	1.64	1.23
X ± SD	0.30 ± 0.05	1.52 ± 0.14	1.10 ± 0.10	1.39 ± 0.09	1.36 ± 0.08	1.30 ± 0.08	1.60 ± 0.10	1.23 ± 0.04

TABLE 4. Relationship between protein intake and EGR-AC of lactating mothers.

Balance Period	Protein Intake (g)	Riboflavin Intake (mg)	EGR-AC
1	54 ± 4	0.03 ± 0.05	1.52 ± 0.14
2	68 ± 4	1.10 ± 0.10	1.39 ± 0.09
3	82 ± 4	1.36 ± 0.08	1.30 ± 0.08
4	96 ± 4	1.60 ± 0.10	1.23 ± 0.04

TABLE 5. Regression of EGR-AC on riboflavin intakes and estimates of riboflavin requirement for an EGR-Ac of <1.3 for lactating women.

Subject	Regression of EGR-AC (y) on riboflavin intake (x) y = a + bx	Correlation Coefficient r	Estimate of Riboflavin Requirement mg/1000 kcal
ROSE	y = 1.80612 - 0.78831 x	0.99	0.65
TESS	y = 1.82451 - 0.80131 x	0.97	0.67
LOR	y = 1.38415 - 0.17607 x	0.97	0.53
REA	y = 1.42678 - 0.24295 x	0.94	0.56
LUCY	y = 1.61634 - 0.54117 x	0.99	0.60
LUZ	y = 1.62892 - 0.53098 x	1.00	0.64
TESS	y = 1.53000 - 0.40896 x	0.91	0.59
FLOR	y = 1.84774 - 0.85852 x	0.87	0.65
MERCY	y = 1.50796 - 0.31616 x	0.89	0.69
BABY	y = 1.43565 - 0.31152 x	0.88	0.47
ELI	y = 1.48849 - 0.35015 x	0.98	0.57
$\bar{X} \pm SD$			0.06 ± 0.07
Range			0.47 to 0.69

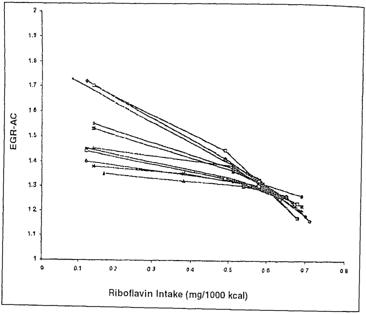

Figure 1. Riboflavin Intake and EGR-AC Values for Lactating Women.

TOXICITY AND IGR EFFECT OF TWO NEEM EXTRACTS ON *MUSCA DOMESTICA* (PCSIR STRAIN)

R. TABASSUM, S.M. NARULAIN, S.N.H. NAQVI
and M.A. AZMI

Department of Zoology, University of Karachi, Pakistan

ABSTRACT

Toxicity of two different neem extracts (N-6a and N-6b) was determined against 3rd instar larvae of Musca domestica *L. (PCSIR strain) and the effect of these extracts on the development was observed.* LC_{50} *of N-6a and N-6b was found to be 18.0 mg/g and 3.6 mg/g medium, respectively. The different doses produced abnormalities in larvae, pupae and adults. At lower dose (0.9375 mg of N-6a) pupation was 76% and emergence was 50% while at higher dose (15 mg/g) pupation was 40% and emergence was 26% indicating IGR effect. At lower dose (0.25 mg/g) N-6b pupation was 42% and emergence was 20% whereas at (61 mg/ g) higher concentration the pupation was 32% and emergence was 16%.*

INTRODUCTION

Various neem products play an important role in the population control of many insect species. Reports given by a number of researchers revealed that the neem in one way causes the death of adults and larvae and in other way we can deter the growth and feeding behaviour of the pest. Rembold and Schmutterer (1981) reported that azadirachtin (isolated from the methanolic extract of the neem seed) is a potent growth inhibitor with only weak antifeedant activity and no acute toxicity against *Epilachna varivestis* larvae even the low dose prevents larval-pupal moulting and blocks further metamorphosis. Steets and Schmutterer (1975), Qadri and Narsaiah (1978), Schluter (1981), Jotwani and Srivastava (1984), Naqvi (1987), Khan et al. (1991) and Tabassum et al. (1992) have also reported IGR effect and toxicity of various neem products. Scores of reports are available on the potentiality of neem as IGR's and toxicant. Besides the above mentioned reports some more notable ones are Maurer (1984), Akhtar et al. (1987), Bidmon et al. (1987) and Wilps (1987). In view of the above findings present authors conducted a study on two neem extracts (N-6a and N-6b) produced locally against 3rd instar larvae of Musca domestica L. The objective of this study was to find an effective IGR which is less toxic, can be locally produced and is cheaper for hygiene pest control.

MATERIALS AND METHODS

Third instar larvae of *Musca domestica* L. (PCSIR strain) were used for the experiments. N-6a and N-6b, the methanolic extracts of the sun-dried coat and kernel of ripe berries of neem, respectively, were obtained through the courtesy of Dr. Bina Shaheen Siddiqui of H.E.J. Research Institute of Chemistry, University of Karachi. Five

different concentrations (Table 1) were selected after preliminary experiments. Three ml of each concentration were mixed in 5 g of sterile breeding medium in small size plastic bowl. Twenty larvae were released in each plastic bowl. Then bowls were covered with muslin cloth and percent mortalities were counted after 24 hours of treatment. Experiments were replicated 7 times. IGR effect of each concentration was also observed for 7 days and percent larval abnormalities, percent pupation and percent emergence were noted. The results were corrected by Abbot's formula and probit mortality graph drawn to find the LC_{50}.

RESULTS AND DISCUSSION

Present work on the efficacy of two neem fractions revealed significant IGR as well as toxic effect on the 3rd instar larvae of *Musca domestica* L. (PCSIR strain). LC_{50} was determined by plotting 24 hours average mortality values of 7 experiments subjected to probit analysis. Mortality percentiles were also corrected by Abbot's formula. LC_{50} from the graph was found to be 18.0 mg/g medium for N-6a and 3.6 mg/g medium for N-6b (Fig. 1). It means N-6b is more toxic than N-6a. The statistical analysis of toxicity data is given in Table 1.

Both neem fractions produced profound effects on the pupation and emergence and also caused abnormalities in the pupae and emerged imago. The growth disruption effect was found to be directly proportional to the concentration of the compounds.

The lowest tested concentration of N-6a i.e. 0.9375 mg/g medium caused an average of 76% pupation, 50% emergence and the percent abnormality was 60% whereas the higher tested dose, 15 mg/g medium revealed 40% pupation, 26% emergence, and percent abnormality was 20% (Histogram I).

Lowest tested concentration of N-6b (0.25 mg/g medium) caused 42% pupation, 20% emergence and 31% abnormalites whereas higher concentration 4.0 mg/g medium produced 32% pupation, 16% emergence and 45% abnormalities (Histogram II). The percentage of pupation and emergence was found to be directly proportional to dose applied.

The aforementioned results revealed that neem fraction N-6a and N-6b may be used as a toxicant and IGR against 3rd instar larvae of *Musca domestica* L. It is also evident from LC_{50}'s of two compounds that lethal value of both the compounds are quite different i.e. N-6b is about 6 times more toxic than N-6a with LC_{50} values of 3.6 and 18.0 mg/g medium, respectively. Similarly, IGR effect by N-6b was produced at lower dose in comparison to N-6a.

The two neem extracts are apparently similar i.e. crude methanolic neem seed extracts but actually they differ with each other, since one is derived from coat (N-6a) and the other is from kernel of neem (N-6b) and it is evident from many reports that extracts from different parts of neem seed and different isolated fractions of neem show a wide range of variation in their activity, either toxicity or IGR and that variation may be due to different amount of active ingredients present in different parts of seed or the solvent used. Such reports have been published by Schmutterer and Zebitz (1984), Ermel *et al.* (1984, 1987), Sharma *et al.* (1984), Naqvi (1987), Parmar (1987), Sankaram (1987), Singh (1987), Naqvi *et al.* (1989), Nurulain *et al.* (1989), Naqvi *et al.* (1990) and Naqvi *et al.* (1991). As far as toxicity level of these two crude extracts as 18.0 mg/g medium and 3.6 mg/g medium of LC_{50} is concerned,

it is well in accordance with other reports of similar work. For instance Sharma *et al.* (1984) reported that 0.1% of methanol soluble fraction of fresh kernel caused 78% larval mortality of *Mythimna separata*. Maurer (1984) reported LC_{50} value of 1.5 ppm against larvae of *Ephestia kuehniella*. Sombatsiri and Tigvattanont (1984) reported that 0.1% methanolic neem seed kernel extract produced 91.4% larval mortality of *S. littura* in the 3rd instar as compared to 30% mortality of *P. xylostella* in 4th instar. Sombatsiri and Temboonkeat (1987) reported the LC_{50} values of 2nd and 4th instar larvae of the diamondback moth, *Plutella xylostella* (L.), treated with aqueous extracts is 0.84% and 8.6%. Naqvi (1987) reported LD_{50} of NfA against *Musca domestica* (L.) as 1.4 μg/fly, LD_{50} of NfB was found to be 0.5 μg/ *Blatella germanica* (L.) 4th instar nymph, LC_{50} of NfD against *Aedes aegypti* L. larvae was 0.58 ppm, of nimocinolide, 0.625 ppm and of isonimocinolide 0.74 ppm.

As far as IGR effect of two tested neem extract is concerned, both produced abnormalities. The common IGR effects were larval-pupal intermediates, thin, slender or elongated, shrinked, and small size pupae, partial emergence and in some cases adults with short abdomen were seen. The IGR effect of neem is reported in abundance. Some of the related reports are as follows: N.P. Schmutterer (1984) reported that topical application of crude neem seed extracts to 4th instar nymph caused brown colouration of the abdomen and a bleached discolouration as well as the creation of haemolymph bubbles in the thoracic area. Jotwani and Srivastava (1984) reported that when larvae of *Chilo partellus* were treated with neem kernel extract, it resulted in most cases in the formation of larval-pupal intermediates and where this did not happen, the larvae either died during the process of development or the adults emerging from the surviving larvae were abnormal and did not live long enough to produce another generation. Hassler (1984) reported that low concentration of neem seed kernel extract (1-2 ppm MeOH extract) incorporated in diet caused several malformations in the final instar larvae of *Manduca sexta*, like larval-pupal intermediates and unability to hatch into adult. Ascher *et al.* (1984) reported that the methanolic neem seed kernel extract was the most effective in preventing pupation and emergence of adults of *Spodoptera littoralis*. Rao and Subrahmanyam (1986) reported that azadirachtin cause 50% adult moult inhibition at 1.66 μg/g of final instar larvae of *S. gregaria*. Akhter *et al.* (1987) observed shrunk larvae and few deformed adults emerged when a neem extract NfD was incorporated in breeding medium of 3rd instar larvae of *Musca domestica* L. Similar reports have also been given by Naqvi (1987) and Jahan *et al.* (1987). Jacobson (1987) reported that exposure of 3rd instar larvae of facefly, *Musca autumnalis* at the doses of 1.1, 10 or 100 μg azadirachtin caused 100% mortality of larvae or developed pupae. Bidmon *et al.* (1987) reported that flies emerging from pupae of larvae treated with azadirachtin were smaller than control. Their wings, legs and proboscis showed typical malformations and their abdomen was often very short. Islam (1987) reported that 1.0% methanolic neem seed kernel extract treatment to *Dicladispa armigera* reduced the grubs development up to 8% i.e., 92% of nymps were inhibited to become adult whereas the same concentration produced 55% inhibition of *Callosobruchus chinensis* to become adult. Meisner *et al.* (1987) reported the effect of aqueous extract of neem on the development of *Ostrinia nubialis* larvae. They reported that at 0.25% concentration larvae and pupae were not developed at all but at 0.05% only 36.6% larvae and 16% pupae developed. Wilps (1987) found that in *Musca domestica*, larval mortality was not correlated with an increase in azadirachtin consumption, but the eclosion rate dropped continuously. Thin, reduced size pupae in comparison to control were characteristic feature. It may be concluded that neem seed extracts are good IGR agents and maybe used as a cheap source of hygiene pest control agents in Pakistan, where neem is easily available.

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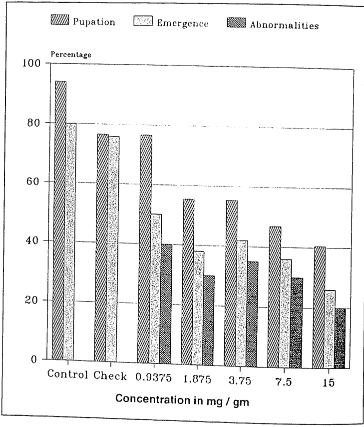
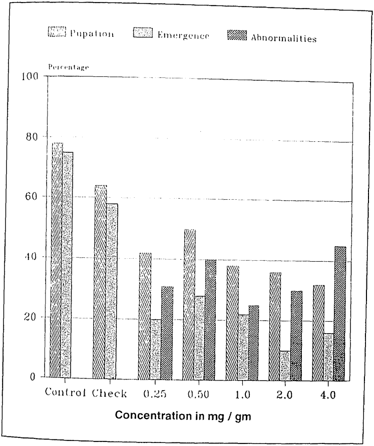
Table 1. Statistical analysis of the toxicity data of N-6a (A) and N-6b (B).

N-6a (A)			N-6b (B)		
Conc. in mg/gm of medium	Average % mortality	95% fiducial limit	Conc. in mg/gm of medium	Average % mortality	95% fiducial limit
Control	4.00±8.94	3.82–11.82	Control	2.00±1.99	1.90–5.90
Check	0.00±0.00	—	Check	8.00±5.39	2.56–18.56
0.9375	16.67±12.24	5.96–27.37	0.25	18.00±5.38	7.45–28.54
0.875	16.67±12.24	5.96–27.37	0.50	24.00±6.40	11.45–36.54
3.750	25.00±4.47	21.10–28.90	1.00	32.00±8.88	14.59–49.40
7.500	35.42±10.95	25.84–45.00	2.00	39.00±9.98	19.44–58.56
15.000	45.83±8.37	38.50–53.16	4.00	67.00±15.09	37.00–96.57

Historgram I: Showing % pupation, emergence and abnormalities after treatment with N-6a.



Historgram II: Showing % pupation, emergence and abnormalities after treatment with N-6b.



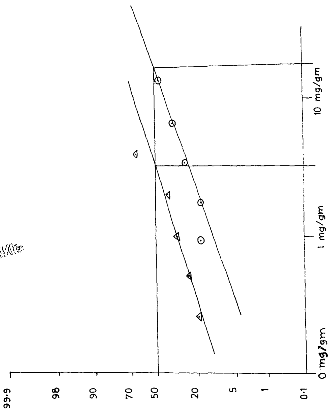


Figure 1. Toxicity curve of N-6a and N-6b against *Musca Domestica* L. (PCSIR strain) LD_{50} of N-6a (△ -△) = 18 mg/gm and N-6b = (⊙ – ⊙) = 3.6 mg

EFFECT OF HEXANE EXTRACT OF *FERULA JAESCHKEANA* ON THE REPRODUCTIVE ORGANS IN CYCLIC FEMALE RATS

A.O. PRAKASH and S. JONATHAN
School of Studies in Zoology,
Jiwaji University, Gwalior 474011, India

ABSTRACT

Hexane extract of Ferula jaeschkeana Vatke has been studied for its effects on reproductive organs in female cyclic rats. Its administration for 10, 20 and 30 days stimulated the uterus as it increased the height of luminal epithelium and number of uterine glands. Ovary exhibited increased number of corpora lutea and absence of matured sized follicles. Extract also caused an increase in the wet weight of ovary and uterus. Amount of total proteins, glycogen and the activity of alkaline phosphatase were altered significantly in ovary and uterus.

INTRODUCTION

Medicinal plants have extensively been investigated for their antifertility activity in female rats (Bhakuni *et al.*, 1970, Prakash and Mathur, 1976, Dhawan *et al.*, 1977, Chaudhury *et al.*, 1981). Of these, large number of them possess mild or potent estrogenic activity when assessed in immature rats (Qureshi and Dixit, 1980, Saxena *et al.*, 1985). Owing to their estrogenic characteristics, these extract may affect the physiology of the reproductive organs. *Ferula jaeschkeana* commonly known as 'Heeng' (family : *Umbellifarae*) has received attention concerning its antifertility activity (Prakash, 1984, 1985 and Singh *et al.*, 1985). In addition to its therapeutic uses (Kirtikar and Basu, 1935, Chopra *et al.*, 1956), Pathak and Prakash (1989) have reported that its hexane extract prevents pregnancy in rats when administered at a dose of 25 mg/kg body weight after coitus (day 1 to 7 post-coitum). Antifertility agents usually exert effects on female reproductive organs, therefore, efforts have been made in the present investigation to assess the effect of hexane extract of *Ferula jaeschkeana* on the physiology and biochemistry of the ovary and uterus of intact cyclic female rats.

MATERIALS AND METHODS

Aerial parts of *Ferula jaeschkeana* were collected from Nullah slopes of Srinagar (Jammu and Kashmir) India and dried under shade. The powdered form of the plant material was extracted with hexane as described earlier (Pathak and Prakash, 1989). The yield of hexane extract was 0.90% w/w in terms of dried starting material. The contraceptive dose of 25 mg/kg was suspended in 5% gum acacia suspended in distilled water (Prakash and Mathur, 1976) and administered orally with an intragastric catheter as per the body weight.

Mature healthy female albino rats of Sprague-Dawley Strain weighing 150+10 g were selected. These animals were housed under uniform husbandry conditions of light (14:10, Light : Dark) and temperature (26+1°C). These animals were allowed free access of food (Gold Mohur rat pellet diet) and water. The vaginal smear of each female rat was examined daily to identify the stage. The rat showing 3-4 normal estrous cycle were selected and randomly distributed into control and experimental. Experimental animals were given the oral administration of hexane extract for 10, 20 and 30 days. For each set parallel control groups were maintained which received vehicle only. After 24 hours of the last treatment with the extract the animals were killed and the ovaries and uteri were excised, freed from adhering tissues, weighed and processed for various biochemical estimations of proteins (Lowry *et al.*, 1951), glycogen (Seifter *et al.*, 1950), acid and alkaline phosphatase activity (Hawk *et al.*, 1954) level of total cholesterol (Zlatkis *et al.*, 1953). Results were analysed statistically using analysis of variance. A small tissue was also fixed in Bouin's fluid and processed for histological studies. Haemotoxylin-eosin stained slides were examined under microscope.

RESULTS AND DISCUSSION

Ponderal Changes

Table No. 1 depicts the ponderal changes in the ovary and uterus under the influence of the hexane extract. The administration of the extract for 10 days increased significantly the wet weight of uterus (> 0.001). However, it did not change significantly the wet weight of ovary (Vs P > 0.05). Similar changes were observed when the extract was administered for 20 and 30 days.

Biochemical Changes

Protein content

It appeared from table 2 that the administration of the extract for 10 and 20 days showed no change in the protein concentration of ovary and uterus when compared to their respective control (P > 0.05). However, its treatment for 30 days significantly increased protein contents in both ovary and uterus (Vs respective control P > 0.001).

Glycogen content

Table 2 depicts that the administration of hexane extract for 10 days marginally increased glycogen contents in the ovary and the uterus. Its administration for 20 and 30 days significantly increased the level of glycogen in both the organs (Vs P > 0.05 and 0.001). The maximum increase has been observed at 30 days schedule (P < 0.001). Results also revealed a successive increase in the glycogen content of the ovary and uterus from day 10 to 30.

Activity of acid phosphatase

Table 3 reveals that the administration of hexane extract for 10 days significantly increased the activity of acid phosphatase in the ovary and uterus (Vs respective control P < 0.05 and < 0.001). Similarly its administration for 20 and 30 days depicted signifi-

cant increase in the activity of acid phosphatase in both the ovary and uterus ($P < 0.001$). The activity was increased successively and gradually when the treatment was increased from 10 to 30 days.

Activity of alkaline phosphatase

Table 3 reveals that the administration of the extract provoked noteworthy elevation in the activity of alkaline phosphatase. Its administration for 10 and 20 days did not induce any significant change in the ovary ($P > 0.05$), however, in uterus, the activity was significantly increased ($P < 0.001$). The administration of the extract for 30 days significantly increased the activity of alkaline phosphatase in both ovary and uterus (Vs respective control $P < 0.001$). It also appeared that in uterus the activity increased from 10 to 30 days, however, in the ovary, there was insignificant difference at 10 and 20 days schedule.

The level of total cholesterol

Table 4 shows that ovary did not depict appreciable change in the level of total cholesterol. The administration of extract for 10, 20 and 30 days, reduced significantly the level of total cholesterol in the uterus when compared to their respective control groups ($P < 0.001$). However, its administration did not alter significantly the ovarian total cholesterol.

The level of esterified cholesterol

Table 4 reveals that the administration of hexane extract for 10 days showed insignificant change in the level of esterified cholesterol in the ovary. Its administration for 20 and 30 days depicted significant alteration when compared to their respective control ($P < 0.001$). However, the level remained unaltered in the uterus at 10, 20 and 30 days of schedule.

Histological Studies

The ovary

The histological picture of the ovary of control rat at estrus stage displayed normal features which included large number of developing follicles in different stage of their development (Fig. 1). Luteal cells were large with prominent nuclei and appeared secretory (Fig. 2). When hexane extract of *F. jaeschkeana* was administered for 10 days, mature sized follicles with prominent nucleus were observed along with prominent nucleolus and cells of *membrana granulosa* (Fig. 3). Corpora lutea were characterized with the presence of multinucleated luteal cells which showed hypertrophy and vacuolation (Fig. 4). Its administration for 20 days showed the presence of mature follicles, the ovum was surrounded by a thick epithelium and the cells of membrane granulosa were indistinct (Fig. 5). On the contrary, luteal cells were elongated, thickened and showed hypertrophy and vacuolation (Fig. 6). The administration of extract for longer duration of 30 days caused similar changes. In some medium sized follicles cells were not normal as the surrounding tissue was indistinct and granulosa cells were broken at several places and ovum showed degeneration and the luteal cells showed hypertrophy and vacuolation (Fig. 7).

The uterus

The histoarchitecture of the uterus of control intact rat at estrus stage showed normal features (Fig. 8) with tall columnar cell having basal nuclei (Fig. 9). When hexane extract of *F. jaeschkeana* was administered for 10 days, remarkable stimulation occurred in the endometrium and myometrium. Stroma was loose and richly vascularized uterine glands were more in number and tortous (Fig. 10 and 11). Stimulation in uterine histoarchitecture was maintained even at 20 days schedule (Fig. 12). Uterine lumen was increased considerably and epithelium showed too much proliferation. Height of luminal epithelium was increased and the nuclei were placed basally (Fig. 13). Stroma was loose and richly vascularized. Uterine glands were elongated and consisted of elongated columnar cells. When hexane extract was administered for 30 days, the uterus remain stimulated and luminal epithelium showed flagile condition. Stroma was loose and darkly stained (Fig. 14 and 15).

Physiological changes in the reproductive organs of intact and pregnant rats are regulated by the hormones of hypothalamo-hypophyseal origin which are inhibited or stimulated by number of endo and exocrine factors. Feed back mechanism is involved in this regulation. Administration of exogenous female sex steroids like estrogen or progesterone or compounds mimicking such type of activities induced physiological disturbances in the genital tract. In the present investigation, it has been observed that the administration of hexane extract of *Ferula jaeschkeana* caused severe changes in the histological features of ovary. It induced the formation of septa in the stroma which divides the connective tissue into compartments. Graffian follicle showed thick epithelium around the ovum and the cells of membrana granulosa became indistinct. The degenerative changes were observed at 30 days duration where the granulosa cells were broken and ovum showed degeneration. Luteal cells in corpora lutea thickened and showed hypertrophy and vacuolation. Such type of changes are usually induced by a typical estrogenic agent. As extract of *F. jaeschkeana* is known for its estrogenicity (Pathak and Prakash, 1989) it is safely said that the histological changes in the ovary are estrogenic motivated. Similarly, the uterine histological structure are known to be motivated by estrogen which cause an increase in the height of luminal epithelium, vascularity with loose stroma and development of uterine glands (Datta *et al.*, 1968, Karkum and Mehrotra, 1973). Owing to estrogenic activity, the administration of hexane extract of *F. jaeschkeana* caused significant stimulation in uterine histoarchitecture as revealed through increased luminal epithelium, musculature, vascularity and well developed glands. Significant proliferation in the luminal epithelium was observed when the extract was administered for 20 days schedule.

Alteration in the ponderal change of the uterus at different stages of estrous cycle is considered as normal phenomenon but it is strictly estrogen dependent. By the virtue of estrogenic activity number of medicinal plants have also been reported to alter wet weight of reproductive organs (Kholkute *et al.*, 1976, Kaliwal and Rao, 1979, Sharma *et al.*, 1987, Shukla *et al.*, 1988, Tewari, 1988). Interestingly, the extract of *F. jaeschkeana* behaves in a similar way.

A typical estrogen is known to increase the protein synthesis (Davis *et al.*, 1956, Datta *et al.*, 1968), mobilization of glycogen (Gregoire *et al.*, 1967, Demers *et al.*, 1972), activity of alkaline phosphatase (Karkum and Mehrotra, 1973, Prakash, 1979) and level of cholesterol (Moskowitz *et al.*, 1956) in the uterus of rats. However, reports on the activity

of acid phosphatase under estrogenic or progestogenic influence is controversial (Hayashi and Fishman, 1961 and Harris and Cohen, 1951). In the present investigation the administration of hexane extract caused significant increase in proteins, glycogen, alkaline phosphatase and total cholesterol in the uterus of intact rat due to its estrogenic influence.

Increase in the protein contents of uterus under the effect of extract suggests that the physiological response is motivated through the formation of new proteins and it is further strengthened with the fact that total wet weight of uterus is significantly increased. Simultaneously as estrogens are known to increase uterine contractility in the uterus, it is expected that more glycogen is to be needed to release high energy.

Acid phosphatase being a lysosomal enzyme is involved in the process of phagocytosis, autolysis which is required for different reproductive processess like nidation and implantation (Leonard and Knobil, 1950, Wood and Psychoyos, 1967, Woessner, 1973). Its activity has also been reported to increase the cellular permeability which makes the process of endocytosis or exocytosis more fast (Boutselis, 1973). In the present investigation the extract of *F. jaeschkeana* increased significantly the activity of acid and alkaline phosphatase in the ovary and uterus. It is assumed that hexane extract is involved in the metabolism of protein and carbohydrates, phagocytosis simultaneously it may also be involved to increase the permeability of cell.

The level of uterine cholesterol is not much changed during different phases of estrogen cycle (Goswami *et al.*, 1963), the estrogen theraphy decreases the cholesterol level (Rosenman *et al.*, 1952). Later, Moscowitz *et al.* (1956) has reported that 17-β estradiol increased cholesterol level significantly. It has been observed that the administration of hexane extract caused an increase in the level of total cholesterol of ovary which might be related to the increased rats of steroidogenesis in this tissue. Therefore, on the basis of present findings it is concluded that the administration of hexane extract of *F. jaeschkeana* caused significant alterations in the biochemical parameters and histological features of ovary and uterus which are motivated due to its potent estrogenic activity. It is expected that due to these physiological disturbances in the reproductive organs the mated female rats are unable to conceive and elicit antifertility effect.

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Table 1. Effect of hexane extract of *F. jaeschkeana* on the wet weight of ovary and uterus of adult intact rats.

(Values are mean ± S.E. and expressed as mg/100 g body weight. Five animals were taken in each set).

Group No.	Duration (days)	Treatment	Ovary	Uterus
1	10	Control	28.4±1.5	145.2±9.1
		Treated	30.4±1.4	256.2±13.8[a]
2	20	Control	40.1±1.5	142.1±9.01
		Treated	34.2±1.7	328.0±16.4[a]
3	30	Control	31.3±1.6	140.3±8.3
		Treated	36.3±1.9	402.2±19.5[a]

P value versus respective control a < 0.001.

Table 2. Effect of hexane extract of *F. jaeschkeana* on the protein and glycogen contents in the ovary and uterus of adult intact rats.

(Values are mean ± S.E. and expressed as mg/100mg and mg/100 g, respectively. Five animals were used in each set).

Group No.	Duration (days)	Treatment	PROTEIN		GLYCOGEN	
			Ovary	Uterus	Ovary	Uterus
1	10	Control	11.2±0.56	11.1±0.52	354.1±17.7	58.3±2.9
		Treated	12.4±0.6	11.5±0.53	388.9±11.9	65.5±3.3
2	20	Control	11.5±0.57	11.6±0.56	359.4±16.9	54.6±3.2
		Treated	13.3±0.6	13.3±0.7	424.7±17.3[c]	86.9±4.1[a]
3	30	Control	11.8±0.59	11.3±0.4	357±17.0	60.1±3.1
		Treated	16.8±0.8[a]	16.4±0.8[a]	498.2±20.8[a]	132.6±7.3[a]

P value Vs respective control a < 0.001 ; c < 0.05.

Table 3. Effect of hexane extract of *F. jaeschkeana* on the activity of acid and alkaline phosphatase in the ovary and the uterus of adult intact rats.

(Values are mean ± S.E. and expressed as mgP/100g/h. Five animals were used in each set).

Group No.	Duration (days)	Treatment	ACID PHOSPHATASE		ALKALINE PHOSPHATASE	
			Ovary	Uterus	Ovary	Uterus
1	10	Control	230.2±11.5	106.6±5.3	550.2±27.5	360.3±12.9
		Treated	274.5±13.4[c]	172±8.4[a]	590.6±26.6	457.06±16.7[a]
2	20	Control	234.4±9.4	110.2±5.7	556.1±23.9	358.06±15.8
		Treated	289.6±13.6[b]	196.7±8.8[a]	623.5±27.4	461.9±25.0[a]
3	30	Control	228.1±5.19	102±5.1	545.5±27.2	364.1±13.2
		Treated	345.7±13.5[a]	239.8±9.2[a]	716.3±28.1[a]	608.7±24.4

P values versus their respective control a < 0.001
b < 0.02
c < 0.05

Table 4. Effect of hexane extract of *F. jaeschkeana* on the level of total and esterified cholesterol in the ovary and uterus of adult intact rats.

(Values are mean ± S.E. and expressed as mg/100 g. Five animals were used in each set).

Group No.	Duration (days)	Treatment	TOTAL CHOLESTEROL		ESTERIFIED CHOLESTEROL	
			Ovary	Uterus	Ovary	Uterus
1	10	Control	0.523±0.029	0.160±0.008	0.169±0.007	0.037±0.003
		Treated	0.515±0.028	0.147±0.006[a]	0.181±0.008[a]	0.046±0.008
2	20	Control	0.520±0.024	0.162±0.009	0.167±0.005	0.039±0.004
		Treated	0.617±0.033	0.140±0.007[a]	0.197±0.009[a]	0.050±0.009
3	30	Control	0.517±0.029	0.159±0.006	0.171±0.006	0.036±0.002
		Treated	0.696±0.040	0.129±0.006[a]	0.230±0.013[a]	0.042±0.007

P values versus their respective control a < 0.001.

Figure 1. Ovary of control rat showing normal histological features (x 120).

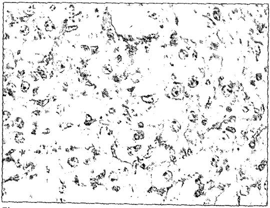

Figure 2. Luteal cells were large with prominent nuclei (x 400).

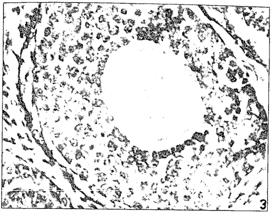

Figure 3. Administration of extract for 10 days showing matured sized follicle (x 400).

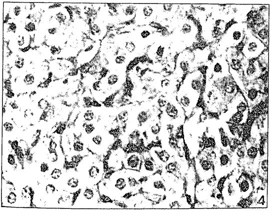

Figure 4. Corpora lutea showing hypertrophy (x 400).

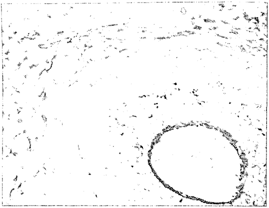

Figure 5. After 20 days of treatment matured sized follicle is surrounded by a thick epithelium (x 400).

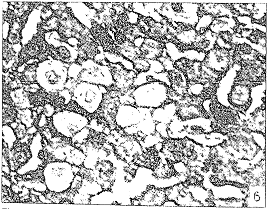

Figure 6. Luteal cells are elongated and appeared secretory (x 400).

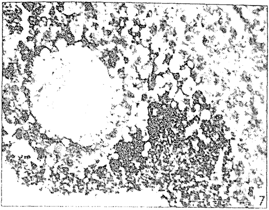

Figure 7. After 30 days of treatment medium sized follicle ovum showed degeneration (x 400).

Figure 8. Uterus of control rat showing normal features (x 120).

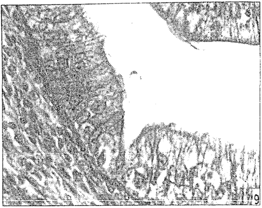

Figure 9. Epithelial cells are tall columnar with basal nuclei (x 400).

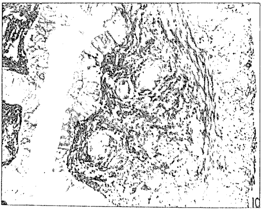

Figure 10. Administration of extract for 10 days Illustrating remarkable stimulation in the luminal epithelium and myometrium (x 120).

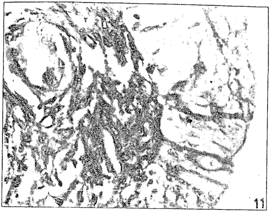

Figure 11. Stroma is loose with tortous uterine glands (x 400).

Figure 12. After 20 days of treatment lumen is widened considerably (x 120).

Figure 13. Additionally the luminal epithelium showed too much proliferation (x 400).

Figure 14. After 30 days of treatment uterus showing stimulated features (x 120).

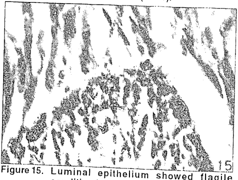

Figure 15. Luminal epithelium showed flagile condition (x 400).

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF PHILIPPINE *CYMBOPOGON CITRATUS* (DC) STAPF

ROSALINDA C. TORRES and ARLENE G. RAGADIO[1]
Chemicals and Minerals Division, Industrial Technology Development Institute (ITDI), Bicutan, Taguig, Metro Manila, Philippines

ABSTRACT

Essential oil from Philippine Cymbopogon citratus (DC) Stapf collected in Nagcarlan, Laguna was obtained from the airdried leaves by hydrodistillation for two hours, and dried using anhydrous sodium sulfate. The oil obtained was then subjected to gas chromatographic analysis. Analysis was done by programming the oven temperature from 70°C - 180°C at 4°C/min for 15 minutes. Components were identified by a peak enrichment method using standard compounds and by comparing GC retention values with those of the standard compounds. Citral was identified as the main component with 69.39% concentration. Geraniol, myrcene, α - and β - pinene, ethyl laurate, 1,8 - cineole, limonene, phellandrene, methyl heptenone, citronellal, linalool, caryophyllene, menthol, terpineol and citronellol were the other constituents detected.

INTRODUCTION

Cymbopogon citratus (DC) Stapf, popularly known as lemongrass grows abundantly in the Philippines. It is a tufted and perennial plant which belongs to the genus *Cymbopogon* of the family *Gramineae* (Quisumbing, 1978). The leaves are up to 1 meter in length, 1.0 to 1.4 cms. wide, scabrous, flat, long-accuminate and glabrous. The panicles are 30 to 80 cms. long, interrupted below. The branchlets and branches are somewhat nodding. The perfect spikelets are linear, lanceolate, pointed, not awned, and about 6 mm. long (Wijesekera).

Lemongrass oil has long been one of the world's best known essential oils and for many years ranked among the most important in terms of the quantities used. It is widely used in a wide range of perfumery products. It is a yellow or amber liquid obtained by distillation of the two species of Cymbopogon: *C. flexuosus* (Steud) Wats. *and C. citratus* (DC) Stapf. The odor of lemongrass oil is basically lemon-like but it also exhibits herbaceous notes not possessed by lemon oils (Robbins, 1983). Several investigations have been done on Philippine lemongrass oil but chemical studies to define its chemical composition was not yet undertaken.

The present study is therefore aimed at obtaining a detailed knowledge about the composition of a hydrodistilled essential oil from *Cymbopogon citratus* (DC) Stapf growing abundantly in the Philippines.

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[1]Standards and Testing Division, ITDI, Bicutan, Taguig, M.M.

MATERIALS AND METHODS

The Plant Material

Matured leaves of *Cymbopogon citratus* (DC) Stapf, commonly known as lemongrass, were collected from Nagcarlan, Laguna, Philippines, a province south of Manila. The plant identity was authenticated by our botanist, Mrs. Evangeline Monroyo, where a voucher specimen of the plant material is deposited at the Pharmaceutical Chemicals Section - Chemicals and Mineral Division, Industrial Technology Development Institute.

The leaves were sorted. All wilted leaves and weeds were discarded. These were then air-dried for seven days. The dried leaves were cut into small pieces, about an inch long, ready for volatile oil extraction.

Isolation and Analysis of Essential Oil

The essential oil was obtained from the air-dried leaves of *Cymbopogon citratus* (DC) Stapf by hydrodistillation for two hours and dried with anhydrous sodium suflate. The oil obtained was then subjected to gas chromatography using standard operating procedure on essential oils, but with some modifications on the temperature of the column. A Shimadzu GC 14A model and a CBP-20 wall-coated fused silica column were used. Temperature programming was performed with the following parameters:

Column temperature	:	70°C, 4 mins. to 180°C, 15 mins.
Program rate	:	4°C/min.
Injection port temp.	:	230°C
Detector	:	FID
Detector temperature	:	230°C
Carrier gas	:	Helium
Inlet pressure	:	0.25 kg/cm2
Sample size	:	0.2 uL neat sample
Injection split ratio	:	1:50

The individual constituents were identified by peak enrichment method using standard compounds and by comparing GC retention values with those of the standard compounds. Standard compounds known as common constituents of lemongrass oil were obtained from Himmel Industries. These were the caryophyllene, 1,8 - cineole, citronellal, citral, citronellol, geraniol, menthol, methyl heptenone, myrcene, linalool, limonene, α - and β - pinene, terpineol, ethyl laurate and phellandrene. These compounds were gas chromatographed at similar conditions.

RESULTS AND DISCUSSIONS

Lemongrass leaves were air-dried for one week prior to distillation. Previous study showed that lemongrass leaves air-dried for one week and containing 9.82% moisture gave the highest yield of 1.40% using water distillation (Coronel, 1984). Lem-

ongrass oil was obtained after two hours of distillation. Distilled oils were collected and treated with anhydrous sodium sulfate. Afterwards, the oil was decanted and placed in another clean vial was now subjected for GC analysis.

The gas chromatogram of the essential oil of Cymbopogon citratus (DC) Stapf is shown in Fig. 1. Identification of the individual components was done by peak enrichment method and by comparing GC retention values of the standard compounds. The concentration in % of the major components found in the oil is illustrated in Fig. 2.

From the results obtained, it was found out that citral was the major component with 69.39%. The strong odor of lemongrass oil with a basically lemon-like character is actually ascribed to the aldehyde citral. The aldehyde content expressed as citral is one of the main criteria for the qualilty assessment of lemongrass oil as specified by the International Organization for Standardization. Most commercial oil contain between 75% and 90% aldehydes, and a high value is desired if the oil is intended for fractionation and isolation of citral.

Philippine C. citratus contains a relatively high value of citral (69.39%) as compared to the Ethiopian (13.5%). The latter's main component is geraniol (40%) (Abegaz, 1983).

The second major component of Phil. lemongrass oil was myrcene with 5.20%. This is an olefinic terpene in the foreruns, which on exposure to air and light, readily polymerizes. The presence of this compound makes the oil less soluble in 70% alcohol (Guenther, 1949).

Geraniol, ethyl laurate, citronellol, terpineol, menthol, caryophyllene, linalool, citronellal, α - pinene, camphene and methyl heptenone were the other constituents present in lemongrass oil. GC also detected the presence of limonene, β - pinene, 1, 8 - cineole and phellandrene but in very low quantities. Four compounds, although present in appreciable quantities were not identified due to the unavailability of other standard compounds. However, the compounds identified constitute over 80% of the oil's total constitution.

ACKNOWLEDGEMENT

We wish to thank the Department of Science and Technology - Region IV and Himmel Industries for providing us the lemongrass samples and essential oil standards, respectively; Messrs. Romulo Estrella and the late Arturo B. Panaglima for the collection of lemongrass leaves and Dr. Beatrice Q. Guevarra for the technical assistance.

Our heartfelt thanks also to Eng'r. Quintillano Montevirgen of the Chemical and Mineral Division for allowing us to use their computer and to the Chemical Process and Development Section, CMD for giving us the much needed assistance in this technical paper.

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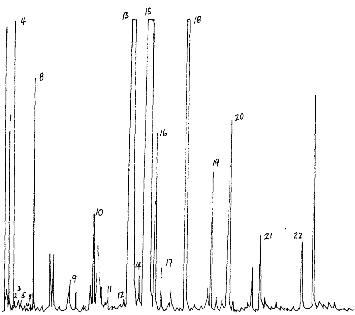
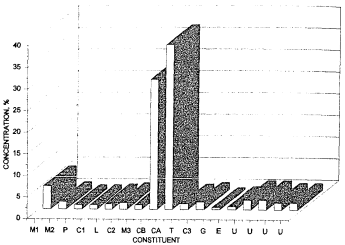


LEGEND:

1. α - pinene
2. β - pinene
3. Camphene
4. Myrcene
5. 1.8-cineole
6. Limonene
7. Phellandrene
8. Methyl heptenone
9. Citronellal
10. Linalool
11. Caryophyllene
12. Menthol
13. Citral b
14. Terpineol
15. Citral a
16. Citronellol
17. Geraniol
18. Ethyl laurate
19. Unidentified
20. Unidentified
21. Unidentified
22. Unidentified

Figure 1. Gas chromatogram of lemongrass oil showing different components as carried out in CBP-20 wall coated fused silica column, Helium gas and detector.



LEGEND:

M 1 - MYRCENE	T - TERPINEOL
M 2 - METHYL HEPTENONE	C 3 - CITRONELLOL
P - α - PINENE	G - GERANIOL
C 1 - CITRONELLAL	E - ETHYL LAURATE
L 1 - LINALOOL	U - UNIDENTIFIED
C 2 - CARYOPHYLLENE	U - UNIDENTIFIED
M 3 - MENTHOL	U - UNIDENTIFIED
C B - CITRAL B	U - UNIDENTIFIED
C A - CITRAL A	

Fig.2. MAJOR COMPONENTS IN LEMONGRASS OIL

Figure 3. Lemongrass plantation in Nagcarlan, Laguna is intercropped with corn and papaya.

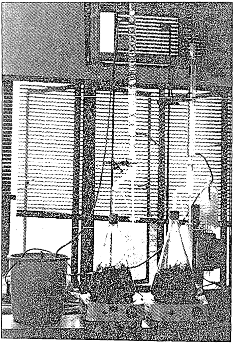


Figure 4. Lemongrass oil is extracted by hydrodistillation from (7) days air-dried leaves.

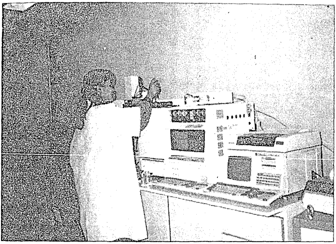


Figure 5. Lemongrass is being injected into the gas chromatographic system.

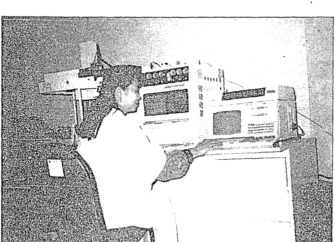


Figure 6. Parameter for the analysis of the chemical composition of lemongrass oil are being entered in the GC software.

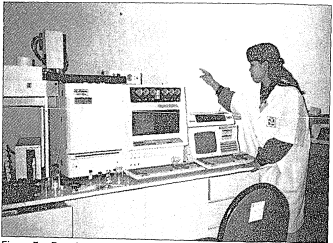


Figure 7. Results of the GC analysis are being scanned over by the researcher.

VALIDATION OF NAMES TRANSFERRED TO KAPPAPHYCUS DOTY FROM EUCHEUMA J. AGARDH (RHODOPHYTA: SOLIERIACEAE)

LAWRENCE M. LIAO
De La Salle University, Taft Ave., Manila

ABSTRACT

Five species of Eucheuma have been recently recognized under the genus Kappaphycus, but the proposed names have remained invalid. Their transfer is effected herein. In addition, the names of two varieties of the generic type, Kappaphycus alvarezii (Doty) Liao, comb. nov. and that of a variety of Eucheuma odontophorum Boergesen are validated.

INTRODUCTION

The economically important algal genera *Eucheuma* J. Agardh and *Kappaphycus* Doty (Rhodophyta: Solieriaceae) are widely distributed in tropical seas. Silva et al. (1987) recorded 15 species of *Eucheuma* from the Philippines. Two of these are farmed extensively in central and southern Philippines for the international carrageenan market. Trono (1992) estimated total Philippine production of carrageenan to be 60,000 MT per year which constitute a little over half of the total world supply. In the course of preparing extensive checklist of economic marine algae from the Cuyo Islands, west central Philippines, extra attention was given to the use of correct names (Liao, 1987). It was soon discovered that a number of species from the area are without validly published names. Such is the case of species recently recognized by Doty (1988) in his new genus *Kappaphycus*. The new binomial combinations formed as a result of the transfer to the new genus unfortunately did not fully satisfy the requirements set by the International Code of Botanical Nomenclature (Greuter et al., 1994) specifies full nomenclatural and bibliographic citation, i.e. basionym, author, year of publication, pagination, figure numbers, if any, as a pre-condition for valid publication of new combinations. In case of species of *Kappaphycus* recognized thus far, names need to be validated. To make validated names available, it is necessary to cite the page omissions of Doty (1988) following the requirements of the Code. This step is imperative as the invalidly published names have already gained wide acceptance and use by researches all over like Glenn and Doty (1990). Li et al. (1990), Santos (1989), Trono (1992), and Trono and Valdestamon (1994), Vreeland et al. (1992), Zablackis et al. (1993) to name a few. None of the works cited above provided the validating information.

TAXONOMIC ACCOUNT

The name *kappaphycus* Doty (1988) in itself is legitimate, having complied with the minimum requirements set by the Code. The designated type is hereby validated as:

Kappaphycus alvarezii (Doty) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma alvarezii* Doty 1985: 37, figs. 1-6.

The two varieties of *E. alvarezii* recognized by Doty (1985) are transfered accordingly to *Kappaphycus*. The correct Latin suffixes are used as suggested by Silva et al. (1987).

Kappaphycus alvarezii var. *tambalangii* (Doty) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma alvarezii* var. *tambalangii* Doty 1985:41, fig. 7 [as *"tambalang"*].

Kappaphycus alvarezii var. *ajakii-assii* (Doty) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma alvarezii* var. *ajakii-assii* Doty 1985: 42, fig. 8 [as *"ajak-assi"*].

Other species of *Eucheuma* recognized under *Kappaphycus* include:
Kappaphycus inerme (Schmitz) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma inerme* Schmitz 1895:150.

Kappaphycus striatum (Schmitz) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma striatum* Schmitz 1895:151.

Kappaphycus procusteanum (Kraft) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma procusteanum* Kraft 1970:215, figs. 1-7.

Kappaphycus cottonii (Weber-van Bosse) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma cottonii* Weber-van Bosse 1913:115, pl. 12, fig. 2.

In addition, an East African taxon of *Eucheuma* needs to be validated:
Eucheuma odontophorum var. mauritianum (Boergesen) L.M. Liao, **comb. nov.**
Basionym: *Eucheuma speciosum* var. *mauritianum* Boergesen 1943: 50, figs 21, 22 [as *"mauritiana"*].

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ADDENDUM

ON THE MORPHOLOGICAL APPEARANCE OF BACCIGEROIDES HAFEEZULLAI (MANNA and DATTA, 1996)

In the 1996 first quarter issue of the Philippine Journal of Science (Vol. 125 No. 1 pp.53-57), a report on a new genus *Baccigeroides* and a new species *B. hafzullai* was published.

Since the observation is new to science and a new genus has been erected with description of a type species *B. hafeezullai*, the appearance is very much essential.

In this issue a photograph of *B. hafeezullai* is appended (see Figure 1).

Reference:

Manna, B. and Datta, I.B. PJS V125(1) 1996. On *Baccigeroides* Gen. Nov. (Digenea: Fellodistomatidae:*Baccigerinae*) in the Intestine of Setipina phasa (Engraulidae) at Chilka Lagoon of Orissa Coast, India.

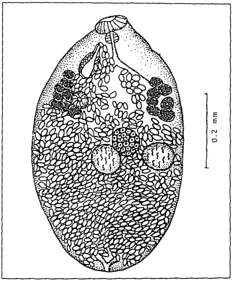

Figure 1. *Baccigeroides hafeezullai* n. gen.; n. sp.. (Camera lucida drawing, ventral view).

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   f. Results and Discussion

   g. Summary/Conclusions/Recommendations (as needed) - to contain an enumeration of the major findings/conclusions/recommendations.

   h. Acknowledgment (if any)

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   Examples: Velasquez, G.T. 1979. The microscopic algae in the hard coral communities. Philipp. J. Sci. 108(3-4): 121-135.

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Example: Kintanar, Q. 1969. Studies on the mechanism on the fatty liver and the hypolipidemia induced by orotic acid in the rat. Ph. D. Thesis. John Hopkins University, Baltimore Maryland, U.S.A.

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Mass	
kilogram	kg
gram	g
ton (metric ton)	t
milligram	mg
Time (same units used in both Metric and English System)	
day	d
hour	h
minute	min
second	s
Amount of substance	
mode	mole
Temperature	
degree celsius	^{o}C